

Current Concepts of Sodium Chloride and Water Transport by the Mammalian Nephron

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FOREWORD

The decision of the editors to solicit a review for the Medical Progress series of this journal devoted to current concepts of the renal handling of salt and water is sound in that this important topic in kidney physiology has recently been the object of a number of new, exciting and, in some instances, quite unexpected insights into the mechanisms governing sodium excretion. These developments have come about largely as a consequence of the fact that segments of nephrons previously inaccessible to direct study are now readily accessible.

Many of the findings to be discussed argue for extensive revision of a number of our current widely held views concerning the renal handling of sodium chloride and water. In the opinion of the authors, the strength of this argument rests in the fact that many of these new findings were obtained under circumstances that enabled workers to gain more direct access to the nephron than has been possible heretofore. This is not to say that areas of controversy and disagreement no longer exist. Wherever possible, these have been identified. In attempting to provide a comprehensive review of this topic, it has been necessary at times to overgeneralize and to disregard minor deficiencies in some of the studies cited. Finally, we wish to emphasize that a considerable portion of the information contained herein derives from work still under active investigation. Much of this contemporary work will undoubtedly withstand the rigors of future experimental scrutiny. It is inevitable, however, as William James so aptly noted in the quotation cited below, that some of our present ideas will need to be abandoned or revised in favor of newer, more convincing evidence. Seen in this light, the present effort is intended as nothing more than a timely survey of this active and fertile topic in renal physiology.

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Science like life feeds on its own decay. New facts burn old rules; then newly developed concepts bind old and new together into a reconciling law.

—WILLIAM JAMES

Glomerulus

A. N. Richards and associates^{1,2} and Walker³ provided experimental verification of Carl Ludwig's more than century-old hypothesis⁴ that the initial step in the process of urine formation involves the elaboration of a nearly ideal ultrafiltrate of plasma across the walls of the glomerular capillaries. These studies, demonstrating that the fluid in Bowman's space has essentially the same composition as plasma with respect to water and low molecular weight solutes, were confined to frogs and necturi, species of amphibia in which glomeruli and surrounding Bowman's capsules are readily accessible to micropuncture. Few such direct measurements of the composition of fluid in Bowman's space have been performed in the mammalian kidney owing to the infrequent presence of glomeruli as surface structures in the mammal. On those rare occasions, however, when surface glomeruli were encountered in rat, guinea pig, and opossum, Walker, Bott, Oliver, and MacDowell⁵ were successful in establishing that, for these species as well, the composition of fluid transported across the glomerular capillary conforms closely to that of an ideal ultrafiltrate.

Ludwig's contribution to our understanding of glomerular function derives not only from his prediction that the fluid in Bowman's space has the characteristics of a nearly ideal ultrafiltrate, but extends also to a qualitative description of the transcapillary forces responsible for the formation of this ultrafiltrate.⁴ Thus, in suggesting that glomerular capillary hydrostatic pressure normally exceeds the sum of the opposing pressures (which he viewed as being determined primarily by what has come to be known as the colloid osmotic pressure of the capillary blood), Ludwig laid the basis for the more quantitative formation by Starling^{6,7} that the magnitude and direction of fluid movement across a capillary wall is governed largely by the algebraic sum of the transcapillary hydrostatic and colloid osmotic pressure differences.

Stated in terms of this familiar Starling relationship, the rate of formation of ultrafiltrate across capillary walls of a single glomerulus is given by the expression:

$$\text{SNGFR} = K_t [(\bar{P}_{GC} - P_T) - (\bar{\pi}_{GC} - \pi_T)]^*$$

where SNGFR is the single nephron glomerular filtration rate, K_t , the ultrafiltration coefficient, \bar{P}_{GC} and P_T , mean glomerular capillary and proximal tubule hydrostatic pressures, and $\bar{\pi}_{GC}$ and π_T , the corresponding colloid osmotic pressures. Whereas a proportional relationship between ultrafiltration rate and net transcapillary driving force has been demonstrated experimentally for capillaries in the mesentery, omentum, and skeletal muscles of a number of amphibian and mammalian species,⁸⁻¹¹ direct evidence in support of a similar relationship for glomerular capillaries has been confined, until recently, only to amphibia.^{12,13}

In the past few years, however, an important discovery and a series of technological advances have enabled workers to acquire similarly direct evidence in the mammalian kidney.¹⁴ Principal among these has been the discovery in the laboratory of Dr. Klaus Thurnau of the Physiological Institute, Munich, of a unique strain of Wistar rats endowed with glomeruli situated on the renal cortical surface.¹⁴ Using these rats, Brenner, Troy, and Daugharty¹⁴ found mean glomerular capillary hydrostatic pressure (\bar{P}_{GC}) to average approximately 45 mm of mercury, or some 40 percent of the mean aortic pressure. It is important to note that the value of 45 mm of mercury for \bar{P}_{GC} obtained with these direct methods is considerably lower than that generally estimated for the rat using indirect techniques such as single nephron stop-flow or ureteral occlusion.¹⁵⁻¹⁷ An explanation for this disparity between direct and stop-flow measurements is provided by the recent finding that the hydrostatic pressure in single capillaries of accessible glomeruli tends to increase, often substantially, when the flow of tubule fluid is stopped.¹⁸ The higher pressures during stop-flow are probably the consequence of compensatory vasodilation of the afferent arteriole in response to elevations in proximal tubule pressure induced by stop-flow.

The lower values of \bar{P}_{GC} obtained by direct measurement indicate that the net driving force for ultrafiltration is also considerably lower than previously thought. Chart 1 summarizes the net

*This equation is referred to elsewhere in this article as Equation 1.

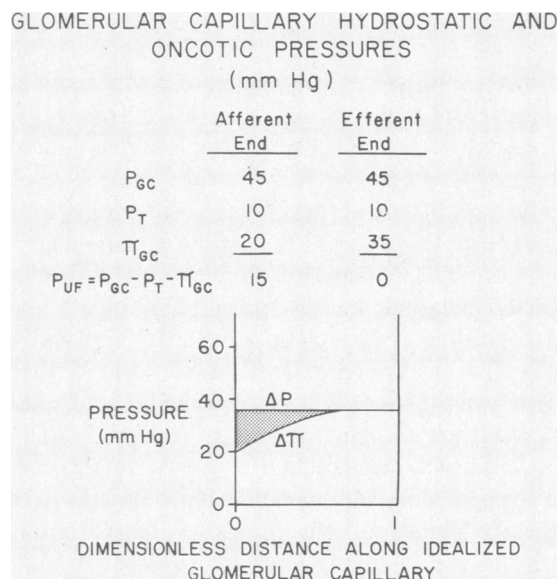


Chart 1.—Hydrostatic and colloid osmotic pressure profiles along an idealized glomerular capillary. $\Delta P = P_{GC} - P_T$ and $\Delta\pi = \pi_{GC} - \pi_T$, where P_{GC} and P_T are the hydrostatic pressures in the glomerular capillary and Bowman's space, respectively, and π_{GC} and π_T the corresponding colloid osmotic pressures.

pressures inferred from these direct measurements. Here the abscissa represents the normalized distance along an idealized glomerular capillary, zero being the afferent end and 1 the efferent end. The local value of glomerular capillary hydrostatic pressure (P_{GC}) is inferred to be the same at afferent and efferent ends of the capillary, about 45 mm of mercury, and P_T , the opposing proximal tubule pressure, is about 10 mm of mercury. ΔP , the difference between P_{GC} and P_T , or 35 mm of mercury, is therefore essentially constant along the length of the capillary, in accord with experimental findings.^{14,19} Measurements of afferent and efferent arteriolar plasma protein concentrations indicate that the colloid osmotic pressure, π_{GC} , increases from about 20 mm of mercury at the afferent end of the glomerular capillary to 35 mm at the efferent end, due to the largely protein-free composition of the ultrafiltrate.^{14,19} Since the protein concentration of ultrafiltrate is very close to zero, π_T is negligible and $\Delta\pi = \pi_{GC}$. Therefore, the local net ultrafiltration pressure (P_{UF}), which is given by the expression

$$P_{UF} = P_{GC} - P_T - \pi_{GC}$$

declines from a maximum value of some 15 mm of mercury at the afferent end of the capillary to zero by the efferent end. As can be seen in Chart 1, this decline in P_{UF} is the consequence

primarily of the rise in $\Delta\pi$. In other words, it has been shown that by the efferent end of the capillary $\Delta\pi$ rises to a value which, on average, exactly equals ΔP .^{14,19-21} This equality of ΔP and $\Delta\pi$ is referred to as filtration pressure equilibrium. To summarize the results of these new direct pressure measurements: (1) ΔP is much lower than previously believed; (2) the finding that $\Delta P = \Delta\pi$ by the efferent end of capillary suggests that some portion of the total capillary does not normally contribute to net ultrafiltrate formation; and (3) since the area between the ΔP and $\Delta\pi$ curves (the shaded area in Chart 1) is equal to the mean ultrafiltration pressure, these recent measurements indicate that the mean ultrafiltration pressure is much lower than was previously thought, averaging only 5 to 6 mm of mercury in the normally hydrated rat, rather than earlier estimates of 25 to 35 mm. Of interest in this regard, Maddox and co-workers recently obtained evidence²² to indicate that the situation in primates is much like that described for rats. Based on similarly direct measurements in squirrel monkeys with accessible surface glomeruli, the evidence indicates that the glomerular transcapillary pressures are virtually the same as in mutant Wistar rats, and that filtration pressure equilibrium is also achieved.

In studies in rats designed to determine how glomerular transcapillary pressures vary so as to bring about changes in SNGFR, evidence has been obtained to indicate that such changes in SNGFR result largely from variations in the rate of rise of $\Delta\pi$, rather than from large or important changes in ΔP .¹⁹⁻²¹ Indeed, ΔP appears to be quite effectively autoregulated.²⁰ Changes in the contour of the $\Delta\pi$ curve arise as a result of changes in the rate of glomerular plasma flow, with increases in the latter tending to displace the point at which filtration pressure equilibrium is reached further to the efferent end of the capillary. The area between the ΔP and $\Delta\pi$ curves is thus increased and the resulting increase in mean ultrafiltration pressure (the area between the ΔP and $\Delta\pi$ curves) brings about an increase in SNGFR. A reduction in glomerular plasma flow has the opposite effect, namely, to displace the equilibrium point further to the afferent end of the capillary, thereby reducing the area between the ΔP and $\Delta\pi$ curves. The resulting reduction in mean ultrafiltration pressure is thereby responsible for a reduction in SNGFR. In general, these observations indicate that the process of glomer-

ular ultrafiltration is highly plasma-flow dependent.

The availability of these direct measurements of glomerular transcapillary pressures and flows, heretofore unavailable in the mammalian kidney, has furnished the incentive for developing a complementary theoretical model of the process of glomerular ultrafiltration.²³ In accord with experimental observations,¹⁹⁻²¹ the model predicts that changes in SNGFR may be viewed almost entirely as a consequence of changes in glomerular plasma flow rate. Furthermore, the model has been found useful in suggesting experiments²¹ that have allowed estimation of the rat glomerular capillary ultrafiltration coefficient (K_f in Equation 1), the product of effective hydraulic permeability and capillary surface area. K_f has been found to be relatively insensitive to changes in glomerular plasma flow rate and the glomerular effective hydraulic conductivity has been determined to be some 50 times larger than that previously reported.²¹

Proximal Tubule

About two-thirds of the glomerular ultrafiltrate is normally reabsorbed by the proximal tubule.²⁴⁻²⁶ In this reabsorptive process, the tubule fluid remains isosmotic with respect to plasma, and there is usually no transepithelial concentration gradient established for Na^+ .²⁵⁻²⁹ Under certain conditions, however, it can be demonstrated that Na^+ reabsorption can occur against an imposed concentration gradient (as during mannitol infusion, which has the effect of lowering the luminal Na^+ concentration to some $30 \text{ mEq}\cdot\text{L}^{-1}$ below that of plasma), suggesting that Na^+ is actively transported out of this segment.^{25,30-33}

That proximal Na^+ reabsorption is an active, energy-dependent process is further supported by recent findings indicating that the proximal tubule of the mammalian nephron can sustain a small but significant transepithelial electrical potential difference (PD) of approximately 2 to 6 mV, lumen negative.³⁴⁻³⁹ For Na^+ reabsorption to proceed against this electrical potential gradient, metabolic energy must be expended. Indeed, the generation and maintenance of this transepithelial PD depends on this expenditure of energy, a conclusion based on the findings that the PD reverts to zero when the epithelium is exposed to cold or to ouabain, a known inhibitor of Na^+ transport.³⁶⁻³⁸ Additional support for this conclusion derives

from the recent findings of Kokko³⁹ indicating that in the isolated rabbit proximal tubule studied *in vitro*, this PD appears to be critically dependent upon the presence in the tubule lumen of essential metabolic substrates such as glucose and amino acids.

The results of several recent experiments emphasize the importance of aerobic metabolism⁴⁰⁻⁴² and oxidative phosphorylation⁴²⁻⁴⁴ in the production of metabolic energy, presumably in the form of adenosine triphosphate (ATP) for active Na^+ transport in the proximal tubule. Na^+ - K^+ -activated ATPase, an enzyme thought to be involved in coupling the transfer of energy from ATP to the Na^+ transport mechanism,⁴³⁻⁴⁶ has been shown to be located along the canalicular infoldings at the bases of epithelial cells (so-called basilar infoldings) as well as along the narrow cleft-like channels between cells (the intercellular channels).^{47,48} Indeed, these basilar infoldings appear to contain more than 100 times as much Na^+ - K^+ -ATPase activity as the luminal brush border.⁴⁹

It would appear, therefore, that the enzymatic machinery necessary for Na^+ transport is located along the intercellular and basilar membranes. Diamond and Bossert,⁵⁰ in an extension of the original three-compartment model of Curran and MacIntosh,⁵¹ have proposed that for epithelia which transport Na^+ and H_2O in isotonic proportions (that is, renal proximal tubule, gallbladder, jejunum), the coupling of Na^+ and H_2O movement is mediated by the active transport of Na^+ into the lateral intercellular channels and basal infoldings with H_2O following passively down the imposed osmotic gradient. Cl^- also follows passively, thereby preserving electroneutrality. This process is illustrated schematically in Chart 2. According to this schema, standing osmotic gradients will develop within these paracellular channels, given the assumptions that (1) the apical (luminal) ends of these channels are tightly sealed (so-called tight junctions) and (2) the channels themselves behave as poorly stirred compartments. It may then be reasoned that as Na^+ is transported into these channels, water follows passively but, at least at the apical ends, not at the same rate as that of Na^+ (due to presumed differences in permeability). As a result, a local (apical) hyperosmotic zone or standing osmotic gradient is created. Osmotic H_2O flow (indicated as the stippled area between adjacent cells in Chart 2) is thought to take place along the entire length of the channel. By the time the fluid

NEPHRON TRANSPORT OF FLUID

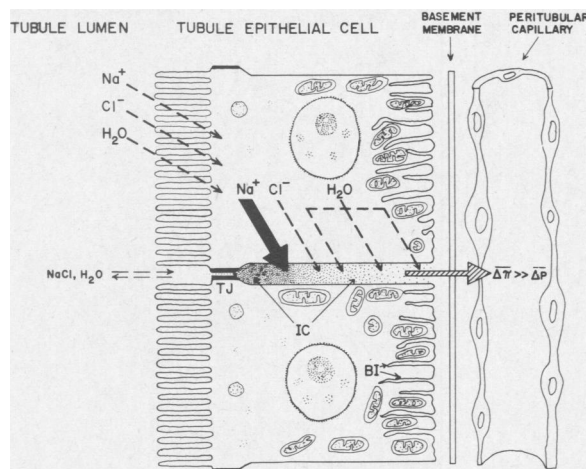


Chart 2.—Idealized proximal tubule epithelial cells under normal hydropenic conditions. (Abbreviations: TJ, tight junction; BI, basilar infoldings; IC, lateral intercellular channel; $\Delta\pi$, mean peritubular transcapillary colloid osmotic pressure difference; ΔP , mean peritubular transcapillary hydrostatic pressure difference.) Solid black arrow denotes active Na^+ transport. Interrupted arrows denote passive transport. Hatched arrow denotes the path for fluid movement as a function of the peritubular transcapillary Starling forces, $\Delta\pi$ and ΔP .

reaches the basilar portion of the channel, sufficient osmotic H_2O flow is thought to occur to yield a reabsorbate that is both isotonic and isosmotic with respect to plasma.

The so-called “tight junction” of all epithelia may not be completely impermeable to salt and water. Indeed, the proximal tubule appears to possess a comparatively “leaky” tight junction.⁵² It has been shown that the net transport rate of Na^+ out of the proximal tubule is only 20 to 30 percent of the unidirectional efflux of this ion,^{29,33} thus indicating extensive back flux due to a high passive permeability of the epithelium to Na^+ . Low resistance paracellular ion shunt pathways have recently been shown to exist across mammalian^{34,53} and amphibian^{54,56} proximal tubules. These have been found to account for as much as 90 to 95 percent of this passive ion movement. The anatomical counterparts of this low resistance pathway are believed to be the “tight junctions” and paracellular channels^{55,56} (Chart 2).

In recent years, kidney physiologists have been concerned to a large extent with efforts to isolate and identify the mechanisms controlling isotonic fluid transport across renal proximal tubules. Two lines of evidence have accrued from these efforts which appear to provide at least a partial understanding of the mechanisms involved. The first

derives from efforts to demonstrate that the control of proximal reabsorption is governed by factors intrinsic to the epithelium of the proximal convoluted tubule. The studies of Burg and Orloff,⁵⁷ Morgan and Berliner,⁵⁸ and Earley, Morel, Ullrich, and their respective co-workers^{29,59-61} convincingly demonstrate that absolute proximal reabsorption changes sufficiently little in response to selective changes in the filtered load of sodium as to suggest that the proximal tubule is endowed with little intrinsic capacity for self-regulation of salt and water transport.

The second line of evidence derives largely from studies that have focused on the role of the peritubular Starling forces in regulating proximal sodium reabsorption. As early as 1843, Ludwig⁴ proposed that passive physical forces might influence net fluid reabsorption by the renal tubule, a suggestion that lay dormant until 1956, when Bresler⁶² similarly theorized that the peritubular capillary colloid osmotic pressure (COP) might provide a purely passive driving force for isotonic reabsorption across this segment of the nephron. In support of this view, Vogel, Heym and Andersohn⁶³ had recently shown that Na^+ reabsorption in the kidneys of amphibians could be enhanced by raising the COP of the peritubular capillary blood. Subsequent studies of Earley, Windhager, Brenner, and their respective co-workers⁶⁴⁻⁷⁶ and others^{38,77-79} have provided additional evidence to support the view that the Starling forces operating across peritubular capillaries exert a strong influence on the reabsorption of salt and water by the proximal tubule.

In order for the oncotic pressure of peritubular capillary plasma to effect the reabsorption of a large fraction of the glomerular filtrate across the proximal tubule by purely passive means, either the transepithelial hydraulic conductivity must be extremely high or the transepithelial osmotic concentration difference must be high, or both. In opposition to these requirements, however, it has been shown that the hydraulic conductivity of the proximal tubule is extremely low,^{33,80,81} and a transepithelial osmotic concentration difference as large as that required (20 to 30 $\text{mOsm}\cdot\text{L}^{-1}$) is not normally found. Therefore, some alternative explanation must be provided to account for this wealth of evidence in favor of a direct relationship between absolute proximal reabsorption and postglomerular COP.

The most attractive explanation to have emerged thus far, that of Lewy and Windhager,⁶⁸

satisfactorily accounts for an action of these physical forces on peritubular capillary uptake and at the same time couples this passive uptake process to the active transport of Na^+ by the proximal tubule epithelium. This coupling is seen to involve the participation of an intermediate compartment (the paracellular channel) situated between tubule lumen and peritubular capillary. Specifically, as shown in Chart 2, Na^+ ions enter the cellular transport pool by passive diffusion down normally existing electrochemical gradients, and are then actively transported into basilar and lateral intercellular channels. Water follows passively, thus elevating pressure in these paracellular channels. This small hydrostatic pressure gradient leads to ultrafiltration of reabsorbate across the tubule basement membrane. The reabsorbate is then moved from the interstitium across the capillary wall, driven by the force defined by the net balance of transcapillary hydrostatic and oncotic pressures. These pressures are denoted in Chart 2 by the symbols $\Delta\bar{P}$ and $\Delta\bar{\pi}$, respectively. Under normal conditions, since $\Delta\bar{\pi}$ greatly exceeds $\Delta\bar{P}$, the net force strongly favors capillary uptake of reabsorbate. In general, any condition that favors a greater increase of $\Delta\bar{\pi}$ over $\Delta\bar{P}$ will result in an increase in net proximal reabsorption. Alternatively, reabsorption will diminish when the balance of transcapillary forces opposes uptake, that is, when $\Delta\bar{\pi}$ no longer greatly exceeds $\Delta\bar{P}$. In the latter case, since capillary uptake of reabsorbate is diminished, egress of fluid at the basilar ends of the paracellular channels is impeded, thereby favoring the passive back flux of ions and water into the tubule lumen. Indeed, under a variety of experimental conditions in which net proximal Na^+ reabsorption is diminished (for example, volume expansion, renal venous occlusion), this passive backflux component for Na^+ has been found to increase dramatically.^{38,56,82,83} The effect of this selective decrease in net peritubular capillary uptake on proximal Na^+ , Cl^- , and H_2O reabsorption is summarized in Chart 3.

Loop of Henle

It is generally accepted that the formation of concentrated urine is the consequence of osmotic equilibration of collecting duct fluid with a hypertonic medullary interstitium. The mechanisms whereby the medullary osmolality is raised are not entirely clear; however, it is thought that the progressive increase in osmolality from the corticomedullary junction to the papillary tip is due

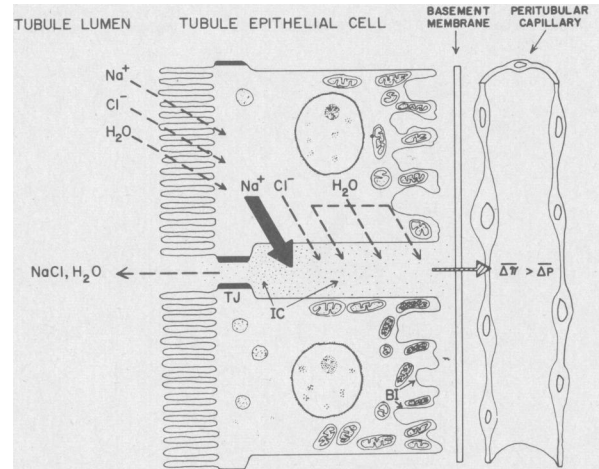


Chart 3.—Idealized proximal tubule epithelial cells under a condition in which peritubular uptake of reabsorbate is diminished (see text). Abbreviations are as given in legend for Chart 2.

to the operation of a countercurrent multiplication system. The operation of the system must have a source of energy. The observation that fluid in the early distal tubule is hypotonic to plasma, while fluid obtained from the bend of the loop is hypertonic, has supported the concept that active outward transport of sodium salts must take place along some portion or all of the ascending limb of Henle's loop.^{25,30,84-86}

To study the nature of the operation of the countercurrent multiplier more directly, experiments were devised to examine the composition of fluid obtained from thin limbs of Henle's loops in the papillary tip region *in vivo*.⁸⁷ This study showed that fluid from thin ascending limbs is lower in osmolality than fluid obtained from thin descending limbs at comparable levels in the papilla. Furthermore, this hypotonicity of the fluid in ascending limbs was found to be due, not to net addition of H_2O ,^{88,89} but rather to net removal of solute, primarily NaCl . No conclusion, however, could be reached from these studies regarding the nature of this salt reabsorptive process from the thin ascending limb. Indeed, because of inaccessibility, it is presently impossible to study entire lengths of thin descending and thin ascending limbs of Henle *in vivo*.

Descending Limb of Henle. Recently, however, using the *in vitro* isolated tubule microperfusion technique of Burg et al,⁹⁰ Kokko and his associates have been able, in rabbits, to examine directly the NaCl , H_2O , and urea transport characteristics of the thin descending limb of Henle,^{91,92} as well as the thin and thick portions

of the ascending limb.⁹³⁻⁹⁵ The thin descending limb was found to be highly permeable to H₂O but to be much less permeable to NaCl and urea. Isotonic fluid enters thin segments of the descending limbs at the corticomedullary junction and equilibrates with the local hypertonic interstitium during its passage toward the papillary tip. Since urea comprises approximately 50 percent of the total osmolality of the papillary interstitium⁹⁶⁻⁹⁸ and since the osmolality of descending limb fluid equilibrates with that of the adjacent interstitium, it would appear to follow that the NaCl concentration of descending limb fluid should be significantly higher than that of the surrounding interstitium. In rabbits, Kokko estimates that this osmotic equilibration process, which has the effect of raising the NaCl concentration to very high levels by the hairpin turn, occurs almost exclusively (96 percent) by water abstraction and to a far lesser degree (4 percent) by solute entry.⁹¹

While a number of investigators have demonstrated net water abstraction from the thin descending limb *in vivo*,^{86-88,99-102} only Kokko⁹¹ has found this to be the overwhelmingly predominant mechanism for achieving maximal osmolality by the hairpin turn. In these studies by others,^{86-88,99-102} solute entry has been found to contribute significantly to the observed elevation in osmolality of tubule fluid by the hairpin turn. Although Kokko has found a high reflection coefficient for urea in *in vitro* perfused thin descending limbs, suggesting little capacity for entry of urea into descending limb fluid, other investigators^{89,99,102,103} have reported significant net urea entry *in vivo*. Explanations to account for these differences have yet to be provided.

Remaining to be explored are the possibilities that these differences in results reflect (1) true species variations (studies showing net solute entry were performed in rats, *Psammomys*, and hamsters,^{89,99,102} while Kokko's studies,⁹¹ showing little net solute entry were performed on rabbit tubules), (2) differences due to comparisons of data obtained *in vitro* and *in vivo*; or (3) the fact that Kokko's studies were performed on descending limbs derived from superficial nephrons, whereas in the *in vivo* papillary tip studies, the nephron segments examined were of juxtamedullary origin.¹⁰⁴

Thin Ascending Limb of Henle. The principal site for active outward transport of NaCl has generally been taken to be the thin ascending limb of Henle's loop. The observation that the fluid

within this segment is hypotonic to fluid in adjacent vasa recta and descending limbs at the same level^{87,88} is consistent with this view. However, using split droplet techniques,¹⁰¹ *in vivo* microperfusion techniques,¹⁰⁵ and *in vitro* microperfusion techniques^{93,94} for studying ascending limbs, no direct evidence has yet been found to prove that this segment is capable of transporting NaCl actively. A concentration of NaCl lower in the thin ascending limb than in the surrounding interstitium could arise, provided that this segment is H₂O impermeable and that its NaCl permeability is great enough to permit the passive outward diffusion of NaCl at a rate sufficient to render hypotonic the fluid remaining behind. In support of this possibility, Kokko⁹⁴ found in *in vitro* microperfusion experiments that the collected perfusate was always lower in NaCl concentration and osmolality than either the initial perfusate or the surrounding bathing medium. Since perfusion fluid and bathing medium had identical osmolalities, whereas the former was relatively rich in NaCl and poor in urea while the reverse was true for the latter, these results are consistent with the view that NaCl can diffuse out of the thin ascending limb more rapidly than urea can diffuse into it. These studies indicate that the thin ascending limb can generate an osmotic gradient, not by active NaCl transport, but rather by purely passive means as a consequence of the differing permeability characteristics of the two thin limbs of Henle to NaCl, urea, and H₂O.

Medullary Thick Ascending Limb of Henle. When the tubule fluid enters the next segment of the nephron, the medullary thick ascending limb of Henle's loop, its relative and absolute amounts of NaCl and urea are lower and higher, respectively, than that of the fluid entering the thin descending limb. The means for lowering NaCl concentration has already been discussed. The elevation in urea concentration appears to take place by two processes: (1) abstraction of water in excess of urea out of the thin descending limb of Henle, and (2) net urea entry (recycled from the collecting duct) into the thin descending and thin ascending limbs of Henle. Using the microperfused isolated rabbit tubule preparation, Kokko⁹⁵ has shown that the electrical PD across the medullary thick ascending limb is oriented lumen-positive (approximately +7 mV). This contrasts with the orientation of the PD along other portions of Henle's loop which are typically lumen-negative.^{106,107} This finding that the lumen of the thick

ascending limb is electropositive, together with the finding that the NaCl concentration continues to decline as fluid travels along this medullary nephron segment, necessitates the conclusion that Cl^- must be the actively transported moiety.⁹⁵ This same conclusion has also been reached by Burg and Green in studies of the isolated cortical thick ascending limb of the rabbit.¹⁰⁸ Since the Na^+ permeability has been found to be greater than that of Cl^- , Cl^- may be regarded as being transported by an electrogenic Cl^- pump while the bulk of the transported Na^+ moves by purely passive means down the existing electrical PD. Besides generating hypotonic tubule fluid, the low water permeability of the thick ascending limb coupled with extensive NaCl efflux gives rise to the well-known high NaCl concentration of the outer medullary interstitium.

Cortical Thick Ascending Limb of Henle. The segment of the thick ascending limb of Henle's loop, which begins at the point where it crosses from the outer medulla into the cortex and extends to the macula densa, is referred to, variously, as the cortical diluting segment, the cortical portion of the thick ascending limb of Henle's loop, or the straight distal tubule. Although the medullary portion of this segment has thicker cells than the cortical portion, the functions of the two parts of the thick ascending limb appear to be similar. As in the case of the medullary thick ascending limb, the cortical thick ascending limb of rabbits has been studied directly by *in vitro* microperfusion techniques.¹⁰⁸ NaCl has been found to be actively reabsorbed across this epithelium of extremely low water permeability (about 1 percent of that of the proximal tubule).

In support of the latter, Burg and Green¹⁰⁸ found essentially no water reabsorption when the fluid perfusing the lumen of this segment was sufficiently dilute to create a large osmotic gradient between tubule lumen and bath, a condition otherwise favoring net water flow. As a result of this absorption of NaCl but not H_2O , luminal fluid becomes hypotonic. This result is in agreement with the observation uniformly obtained in *in vivo* micropuncture studies in a number of species, in that fluid collected from the early distal convoluted tubule has been shown to have low Na^+ concentrations.^{24,26,84}

In further accord with the results of studies of the medullary portion of the thick ascending limb,⁹⁵ Burg and Green¹⁰⁸ found Cl^- transport in the cortical segment of the thick ascending limb

to also be an active, energy-dependent process, since Cl^- concentration decreased in the lumen despite opposing gradients of chemical concentration and electrical potential. The orientation of the electrical PD with lumen-positive across the cortical thick ascending limb results from the active, outward transport of Cl^- and disappears when Cl^- is removed from the lumen and bath, and sulfate or nitrate are substituted.¹⁰⁹ Again as with the medullary portion of the thick ascending limb, Na^+ transport is therefore largely passive.^{108,109}

A Passive Equilibration Model of Countercurrent Multiplication. On the basis of the foregoing, Kokko and Rector¹¹⁰ have proposed a passive equilibration model of countercurrent multiplication to explain the production of concentrated urine. In this model it is envisaged that when the hypotonic fluid leaves the thick ascending limb of Henle's loop and courses through the distal tubule and cortical collecting tubule, it undergoes osmotic equilibration with the surrounding isotonic interstitium by virtue of water abstraction in the presence of antidiuretic hormone (ADH). The permeabilities of the cortical segment of the thick ascending limb of Henle's loop and the distal convoluted tubule to urea are believed to be extremely low. Indeed, Burg et al¹¹¹ have shown that even in the presence of ADH the cortical collecting tubule is completely impermeable to urea. Similar results have been obtained by Schafer and Andreoli¹¹² for the outer medullary collecting duct. Thus, the urea concentration in the fluid entering the cortical collecting duct is extremely high and rises further as the collecting duct passes through the outer medulla. This progressive rise in urea concentration is the consequence of water removal, which, in turn, is secondary to the high NaCl concentration in the surrounding outer medullary interstitium. This high urea concentration in the collecting duct fluid persists until the fluid reaches the inner medulla where, because of a pronounced local *increase* in collecting duct permeability to urea, urea now diffuses down its concentration gradient into the papillary interstitium.¹¹³

To a variable extent, as already discussed, urea is recycled into thin descending and thin ascending limbs. In terms of the passive equilibration model, however, the main function of this high interstitial urea concentration is to exert an osmotic effect on thin descending limbs, favoring abstraction of H_2O and raising the concentration

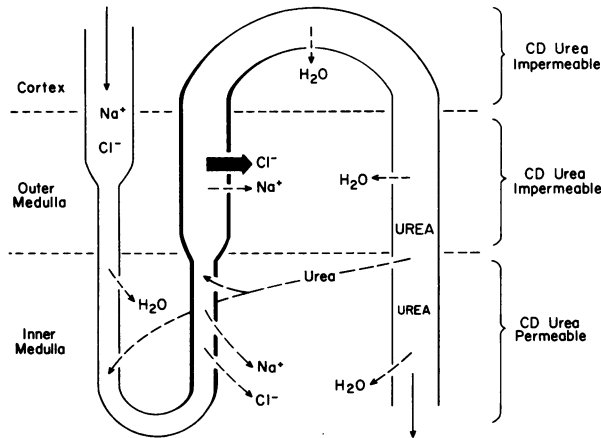


Chart 4.—A schema of countercurrent multiplication by means of passive equilibration of solute in the renal medulla. Solid black arrow denotes active Cl^- transport. Interrupted arrows denote passive transport. The portion of the collecting duct (CD) in the cortex and outer medulla is urea impermeable, whereas the inner medullary CD is highly urea permeable. The solid dark lines along the ascending limb of Henle denote water impermeability. Nephron segments downstream to the ascending limb are shown to be water permeable due to the presence of ADH (modified from Kokko and Rector¹¹⁰).

of intraluminal NaCl . The thin ascending limb, in turn, is presented with a hypertonic fluid rich in NaCl . Because the thin ascending limb is impermeable to H_2O , slightly permeable to urea, and highly permeable to NaCl , the latter can diffuse down concentration gradients at rates faster than the inward diffusion of urea, leading to progressive hypotonicity of the fluid as it flows toward the renal cortex. The combination of these processes could then account for the observed progressive rise in the interstitial osmolality from the corticomedullary junction to the papillary tip. An idealized version of how the limbs of Henle and collecting duct serve to favor passive equilibration of solute in the region of the papilla, as proposed by Kokko and Rector,¹¹⁰ is summarized in Chart 4.

In the intact animal, the fluid that leaves the cortical thick ascending limb of Henle is low in Na^+ and Cl^- concentration, and the concentrations of these ions are largely independent of the animal's state of hydration or diet. Were this fluid to be excreted without further modification, the urine would always be dilute. Usually, however, urine does not have these characteristics but instead its composition is altered further by the action of other transport processes located in more distal nephron segments.

Distal Nephron

The remaining part of the nephron consists of several anatomically and physiologically discrete tubule segments which, together, account for a substantial fraction of the overall reabsorption of NaCl . Control of urinary dilution and concentration (that is, H_2O reabsorption) and the final modulation of net potassium excretion occur in these nephron segments as well. In addition, hormones such as ADH and aldosterone and a number of the clinically useful diuretic drugs are believed to exert major effects here.¹¹⁴

The Distal Convoluted Tubule. This segment is conventionally described as that portion of the nephron extending from the macula densa to its junction with one or more other distal convoluted tubules to form the cortical collecting tubule. This segment has been extensively studied by conventional micropuncture techniques in rats, dogs and monkeys.^{24,26,84,85} In rats, H_2O reabsorption by this segment is variable, depending on the state of hydration and, more specifically, on the presence or absence of ADH.¹¹⁵ In the absence of ADH, this and more distal nephron segments are essentially impermeable to H_2O , so that the hypotonic fluid entering this segment is excreted as dilute urine. Indeed, continued NaCl reabsorption along the distal convoluted tubule results in further dilution of the urine in the absence of ADH. In the presence of ADH in rats, the permeability of the late portion of this segment to H_2O increases greatly. As a result, the osmolality of late distal tubule fluid rises to a value close to that of plasma. There is disagreement among investigators as to whether the fluid equilibrates completely with the surrounding isosmotic interstitium, but all agree that an increase in osmolality toward that of plasma is achieved.¹¹⁶ In dogs^{24,85} and monkeys,²⁶ the situation in the absence of ADH is similar to that described for rats. In the presence of ADH, however, the evidence thus far seems clear that full osmotic equilibration (fluid-to-plasma osmolality ratio of unity) with the surrounding isosmotic interstitium does not take place along the late distal tubule. Instead, fluid-to-plasma osmolality ratios achieve values of approximately 0.8. In dogs and monkeys, and in those groups of rats which fail to reach osmotic equilibration by the late distal convoluted tubule, isosmolality is achieved in the cortical collecting tubule.

The electrolyte transport system in the distal convoluted tubule has been studied extensively.^{84,117,118} It is generally observed that NaCl

continues to be reabsorbed from the tubule lumen.^{24,26,84} In rats, net Na^+ reabsorption proceeds uphill against a moderately steep chemical concentration gradient¹¹⁸ and against an electrical potential gradient which increases from -12 mV (lumen-negative) in the early distal tubule to about -45 mV (lumen-negative) by the late tubule.¹¹⁹ In the absence of aldosterone, the capacity of the epithelium of the distal tubule to lower the Na^+ concentration in tubule fluid has been shown to be reduced.¹²⁰ From calculations using the Nernst equation, which describes the distribution of charged ions across a membrane under conditions of diffusion equilibrium, the transport of Cl^- to a large extent can be ascribed to purely passive forces, namely, diffusion down an electrical PD from tubule lumen to peritubular capillary blood. That Cl^- might also be transported by an active extrusion mechanism in this segment was first suggested by Rector and Clapp.¹²¹ Recent evidence by Malnic and Giebisch¹²² offers further support for this conclusion. As with rats, dogs have recently been shown to be capable of transporting NaCl against steep electrochemical gradients.³⁴

Cortical Collecting Tubule. This segment extends unbranched from the junctions of distal convoluted tubules, through the cortex to the junction with other cortical collecting tubules in the outer medulla. This segment has been the subject of extensive recent investigations using the technique of micropfusion of isolated rabbit tubule fragments.¹²³⁻¹²⁶ These studies demonstrated that this epithelium possesses an extremely low permeability to H_2O in the absence of ADH and that this permeability increases greatly in the presence of this hormone. The sensitivity of this segment to ADH appears to be more pronounced than is that of the distal convoluted tubule. Urea permeability has been found to be low and, as discussed above, to be remarkably insensitive to ADH. This unresponsiveness of urea permeability to ADH is considered essential to the efficient recycling of urea in the operation of the passive equilibration model of urine concentration discussed above.

Studies on the nature of electrolyte transport using micropfusion techniques indicate that this segment is capable of further net reabsorption of NaCl . The rate of Na^+ transport here is considerably lower than that of the proximal convoluted tubule and more like that of the distal convoluted tubule. In micropfusion studies of this segment

in the rabbit, the transepithelial PD is about 35 mV (lumen-negative).¹⁰⁹ By comparing the theoretical PD based on the differences in the concentrations of electrolytes in the luminal fluid and surrounding bathing medium with the PD actually measured,¹²⁵ and by studying the flux ratios for Na^+ and Cl^- ,¹⁰⁹ it appears that Na^+ reabsorption is active and that Cl^- reabsorption can be accounted for entirely by passive means.

Papillary Collecting Duct. The terminal segment of the distal nephron is the highly branched papillary collecting duct. This segment has been studied in rats^{127,128} and hamsters,¹²⁹ both by micropuncture of the surface of the papilla exposed by dissection of the renal pelvis, and also by retrograde catheterization from the tip of the papilla. These more recent direct studies have generally supported earlier conclusions based on comparisons of data obtained from late distal sites with final urine.⁸⁴ Continued electrolyte transport in this segment results in the typically large ion concentration differences that normally exist between urine and plasma. Na^+ transport appears to be active, since reabsorption proceeds against sizable electrochemical gradients.^{129,130} The rate of Na^+ transport in this segment depends on the diet and on the load of sodium delivered from earlier segments¹²⁸ and is affected by the presence or absence of aldosterone.¹³¹ It would also appear that this segment has the ability to effect active Cl^- reabsorption.¹²⁸ Water permeability increases markedly in the presence of ADH,¹³² an effect similar to that seen in the distal convoluted tubule and cortical collecting tubule. Importantly, the papillary collecting duct differs from these latter structures in that its permeability to urea increases dramatically in the presence of ADH. For the reasons noted above, the high H_2O and urea permeability of the papillary collecting duct in the presence of ADH appears to be of fundamental importance to the formation of hypertonic urine.

Thus, there are at least three distinct segments of the distal nephron—the distal convoluted tubule, the cortical collecting tubule, and the papillary collecting duct. The electrical potential differences of these segments vary somewhat, but so far have always been found to be lumen-negative. In each segment there is net NaCl reabsorption. Active Na^+ transport predominates in all segments. Evidence has been cited to indicate that Cl^- can be actively transported from the distal convoluted tubule and papillary collecting duct, whereas in the cortical collecting duct, Cl^- has

been found thus far only to be transported passively. Water permeability is variable in distal nephron segments, depending on the presence or absence of ADH. Urea permeability is low and unaffected by ADH in all segments except the papillary collecting duct, where the increased permeability to urea in the presence of ADH appears to be important in the recycling of urea and the production of hypertonic urine.

Summary

The aim of this review has been to provide a current survey of our present knowledge of the mechanisms governing sodium chloride and water transport across the various anatomical segments of the mammalian nephron. We have dealt more completely with those aspects of renal salt and water transport that have recently yielded new insights (for example, glomerular ultrafiltration, the passive function of the thin limbs of Henle) than with areas of knowledge that have tended to change little in recent years. Limitations of space have precluded extensive discussion of the intrarenal and extrarenal factors governing the control of sodium excretion. Nor have we been able to review the extensive progress that has been made in areas of renin-angiotensin-aldosterone interaction and regulation of regional distribution of renal blood flow. For current thinking in these fields, the reader is referred elsewhere for excellent summaries.¹³³⁻¹⁴²

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